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Purpose: Amplify pGAPDH ~~for cloning and transformation~~ w different enzymes again.

- GAPDH / Regular Forward + Reverse primers / DeepVent worked (pg 97)
- never tried with Taq + DeepVent,
- but no problem with Taq alone (pg 88).

Used: DeepVent buffer - 2 mM Mg — 25 (94°, 30"; 60°, 1'; 72°, 15') 95° 5' 72°, 10'
200 μM dNTP
0.5 μM primer
100 pg template (10 pg/λ)

Taq:		Unit	Taq + DeepVent			DeepVent	
			# 31	32	1U + .001		
# 1	2	0	33	34	.005	# 15	16
3	4	.5	35	36	.01	17	18
5	6	1	37	38	.05	19	20
7	8	1.5	39	40	.1	21	22
9	10	2	41	42	.2	23	24
11	12	2.5	43	44	.5	25	26
13	14	5	29	30	1	27	28
15						45	46
						47	48
	15x		17x				

10x buffer	75
dNTP	15
Template	150
primer 1	3.75
primer 2	3.75

50 μl / Rx

Cocktail → 50 μl / Rx + enzyme dif. amount D.V + 1U Taq in all

20x 50 μl + dif. amount of deepvent

added enzyme separately in 1 μl

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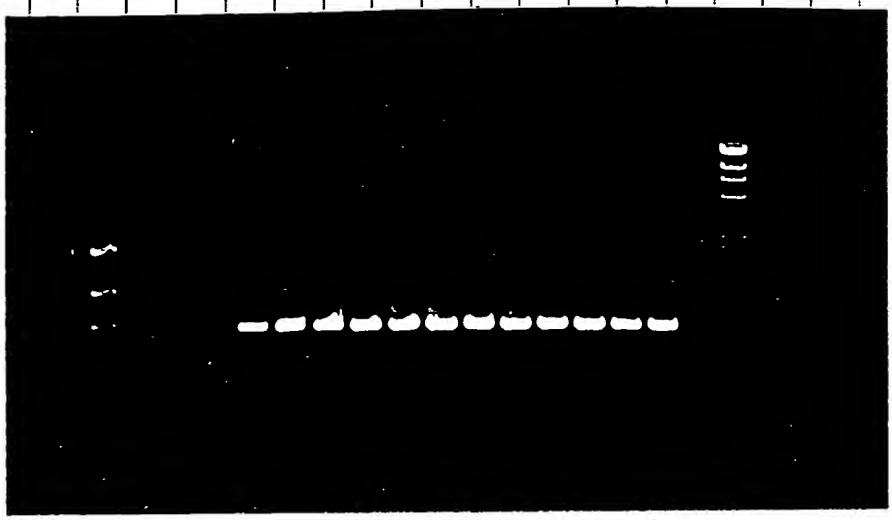
Witnessed & Understood by me, [signature] Date 12/14/94

Invented by

Recorded by K. Sitaraman Date 11/30/94

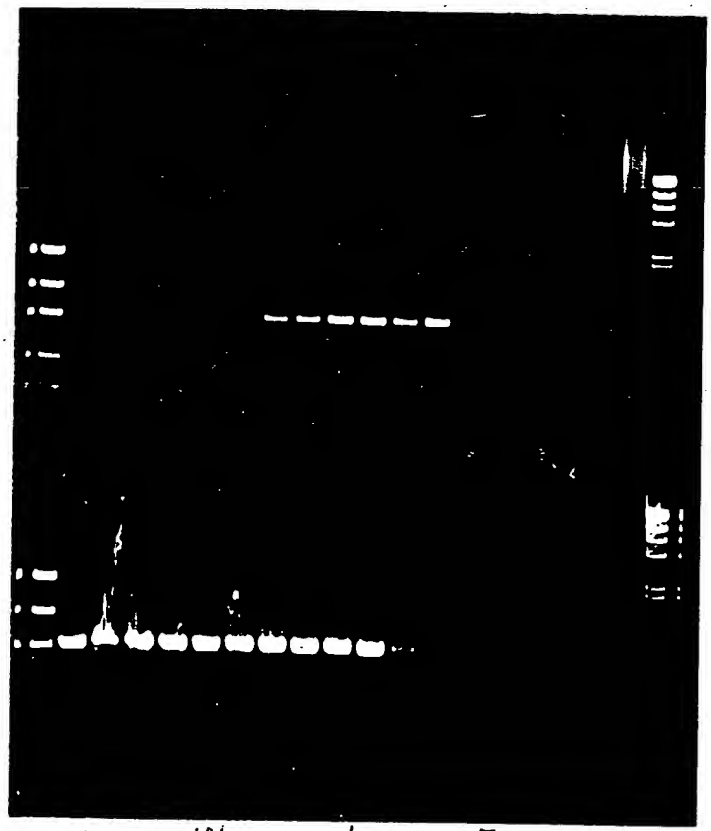
Tag N .__

Taq



0 .5 1 1.5 2 2.5 5

.001 .005 .01 .05 .1 .2 .5 1 2



1U Taq+ .001 .005 .01 .05 .1 .2 .5 1U Deepvent

Result:

- 100 pg + 25 cycles seem to be enough
- Deepvent alone with regular primers worked again
- Deepvent at lower concentration works better. With 0.05 U good product yield was seen.
- So may be worth earlier run, if the enzyme conc is lower, it might have worked with Deepvent. Why?

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sed & Understood by me,	Date 12/19/94	Invented by	Date
		Recorded by K. Sivaraman	12/1/94